Micropapillary Serous Carcinoma of the Ovary Has Distinct Patterns of Chromosomal Imbalances by Comparative Genomic Hybridization Compared With Atypical Proliferative Serous Tumors and Serous Carcinomas

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Recent studies have subdivided serous borderline tumors into 2 categories: atypical proliferative serous tumors (APSTs), which have a relatively benign course, and micropapillary serous carcinomas (MPSCs), which behave like low-grade carcinoma. This study was undertaken to determine, using comparative genomic hybridization (CGH), whether cytogenetic changes support this hypothesis. Nine cases of APST, 10 of MPSC, and 11 of invasive serous carcinoma (SC) were analyzed by CGH. Tumor DNA was extracted from frozen or paraffin-embedded tissue from the primary ovarian tumor, using either sections with at least 70% tumor cells or tissue after relative enrichment by microdissection. Chromosomal imbalances were identified in 3 of 9 APST, 6 of 10 MPSC, and 11 of 11 SC. Three or more chromosomal imbalances were found in 0 of 9 APST, 4 of 10 MPSC, and 9 of 11 SC. Recurrent copy number alterations were grouped into 4 classes correlating with the different tumor types. Class I changes were present in APST and in MPSC or SC and included +8q (7 of 11 SC, 2 of 10 MPSC, 2 of 9 APST), −9p (5 of 11 SC, 0 of 10 MPSC, 1 of 9 APST), and +12 (+12p in 3/11 SC, +12 in 2 of 10 MPSC, +12 in 1 of 9 APST). Class II changes were found only in MPSC and SC, but not in APST. The most frequent examples were +3q (10 of 11 SC, 1 of 10 MPSC), −4q (5 of 11 SC, 1 of 10 MPSC), and −17p (5 of 11 SC, 1 of 10 MPSC). Class III changes were limited to SC, like −16q (7 of 11 SC) and −18q (6 of 11 SC). Class VI changes were unique to MPSC. Gain of 16p (3 of 10 MPSC) was the only aberration in this group. This aberration was not only unique to MPSC but was also the most frequent finding in MPSC. These data support the hypothesis that noninvasive serous tumors of the ovary can be subdivided into 2 categories: APST and MPSC. The number of imbalances in MPSC is substantially higher than in APST and lower than in SC. Some changes in MPSC are shared with SC and APST and others with SC only, suggesting that a subset of MPSC may represent a stage in progression from APST to SC. Other cases of MPSC with independent genetic alterations may represent another subset of tumors that are a distinct entity from APST and SC. HUM PATHOL 33:47-59.

Key words: **chromosomal imbalances, micropapillary serous carcinoma of the ovary.**

Abbreviations: **MPSC, micropapillary serous carcinoma; APST, atypical proliferative serous tumor; CGH, comparative genomic hybridization; dUTP, deoxyuridine triphosphate; SSC, standard saline citrate; TRITC, tetramethylrhodamine isothiocyanate; DAPI, 4,6-diamino-2-phenylindole; ANCA, average number of copy alterations.**

Micropapillary serous carcinoma (MPSC) was first recognized in a review of noninvasive proliferative serous tumors of the ovary, which according to the World Health Organization (WHO) classification qualified as borderline tumors.[1] Based on that study and a subsequent analysis comparing advanced-stage MPSCs with typical serous borderline tumors, MPSCs had a significantly worse prognosis than typical serous borderline tumors. Furthermore, when MPSCs recurred, they had the appearance of bona fide carcinoma.[2] Accordingly, it was proposed that proliferative serous tumors that qualified as borderline by WHO criteria could be divided into 2 groups based on their histologic appearance, MPSC, a low-grade carcinoma, and atypical proliferative serous tumor (APST), a benign tumor. The prognostically less favorable MPSC is characterized by long, thin papillae arising from thick fibrovascular cores. In contrast, atypical proliferative serous tumors show a hierarchical pattern of branching fibrovascular cores with steadily decreasing caliber. This division dissolves the borderline category.

Other investigators confirmed that patients with advanced-stage MPSCs had a 10-year survival rate of approximately 70%, whereas patients with advanced-stage typical borderline tumors, that is to say APSTs, had a survival approaching 100%. However, these investigators preferred to retain the borderline category designating noninvasive serous tumors with a micropapillary architecture as "serous borderline tumors, micropapillary type."[3] To further delineate the relationship of MPSC to APST and invasive serous carcinoma of the ovary, we undertook a genetic analysis using comparative genomic hybridization (CGH).

CGH is a molecular cytogenetic screening test that

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can be performed on DNA extracted from paraffin-embedded tissue without the need for cultivated tumor cells. This technique allows analysis of extracted whole-tumor genomic DNA for relative gains and losses by mapping regions of copy number alterations on normal metaphase chromosomes.[4]

Although there is abundant cytogenetic data on invasive ovarian carcinomas,[5] including more than 100 cases that have been analyzed by CGH,[6-9] few cases of borderline tumors have been analyzed by traditional or molecular cytogenetic techniques.[10-13] These studies have shown that serous borderline tumors have on average significantly less chromosomal alterations than serous carcinomas, and many tumors do not show any alterations at all. However, these studies do not indicate whether tumors with a micropapillary architecture were analyzed. Thus, it is not clear whether MPSCs were included or whether they were analyzed but classified as serous borderline tumors. We undertook the present CGH analysis of noninvasive serous tumors to determine whether the division of serous borderline tumors into 2 categories, MPSC and APST, was justified based on the cytogenetic findings.

MATERIALS AND METHODS

Case Selection

Cases designated as serous borderline tumor or MPSC were identified from the surgical pathology files of The Johns Hopkins Hospital and the consultation files of one of the authors (R.J.K.). The tumors were reviewed by 2 of the authors (A.S. and R.J.K.) and classified as MPSC or APST according to published criteria.[1] In addition, 11 cases of invasive serous carcinoma were selected for CGH.

Microdissection

Ten consecutive sections, 5 μm in thickness, were cut from paraffin blocks or frozen tissue of the primary ovarian tumor. These sections were flanked by hematoxylin and eosin–stained slides to correlate the morphologic and cytogenetic findings. The unstained sections were immersed in xylene twice for 15 minutes each time and then rehydrated in decreasing concentrations of alcohol. After immersion in 70% ethanol, the sections were briefly air dried. The area of interest was soaked in 2.5% glycerol in Tris/EDTA immediately before microdissection to facilitate the pickup and transfer of the material into 100% ethanol. Areas containing at least 75% tumor cells were microdissected under a Zeiss dissection microscope with a 26G needle or a single-use scalpel. The tissue was then kept at −80°C for further processing. After removal of the ethanol, the pellet was resuspended in 1 mL NaSCN (1 mol/L) and incubated overnight at 37°C. DNA was prepared using proteinase K digestion and phenol extraction.

Comparative Genomic Hybridization

CGH was performed on normal female metaphase chromosomes prepared according to standard procedures. Control DNA was labeled by nick translation, substituting deoxythymidine triphosphate by digoxigenin 12-deoxyuridine triphosphate (dUTP; Boehringer Mannheim, Indianapolis, IN). Genomic tumor DNA was labeled with biotin-16-dUTP (Boehringer Mannheim). Two micrograms of control DNA and 2 μg of tumor DNA was precipitated together with an excess (50 μg) of Cot-1 fraction of human DNA (Gibco BRL, Gaithersburg, MD). The pellet was dryed for 5 minutes in a speed vac and resuspended in 10 μl of hybridization solution (50% formamide, 2× standard saline citrate [SSC], 10% dextran sulfate). The probe was denatured for 5 minutes at 75°C and preannealed for 1 hour at 37°C. The normal metaphase chromosomes were denatured separately for 2 minutes at 75°C in 70% formamide, 2× SSC, and dehydrated through an ethanol series. Hybridization took place under a coverslip for 2 to 4 days at 37°C. Posthybridization washes and immunocytochemical detection was performed as described.[14] Biotin-labeled tumor sequences were detected with avidin conjugated to fluorescein isothiocyanate (Vector Laboratories, Burlingame, CA), and the digoxigenin-labeled reference DNA was developed using a mouse antidigoxigenin antibody, followed by a tetramethylrhodamine isothiocyanate (TRITC)-conjugated anti-mouse antibody (Sigma, St Louis, MO). The chromosomes were counterstained with 4,6-diamino-2-phenylindole (DAPI) and embedded in an antifade solution containing paraphenylenediamine (Sigma).

Gray-level images were acquired for each fluorochrome with a cooled charge-coupled device camera (Sensys; Photometrics, Tuscon, AZ) coupled to a Leica DMRXA epifluorescence microscope (Leica Microsystems, Wetzlar, Germany) using sequential exposure through fluorochrome-specific filters. Images were captured with help of the Leica Q-FISH imaging system. Chromosomes were identified using DAPI banding. Fluorescence ratio images were calculated with the Leica Q-CGH software. Average ratio profiles were calculated from at least 9 individual metaphases per case. A chromosomal gain was considered significant if the ratio between tumor and normal DNA was >1.25. A relative loss was scored with a ratio of <0.75. Statistical analysis of the average number of chromosomal aberrations for the individual groups was performed using the *t* test (SPSS; SPSS Inc, Chicago, IL).

RESULTS

The basic clinical and histopathologic data on the 30 cases are summarized in Table 1. Representative examples of APSTs, MPSCs, and serous carcinomas are shown in Fig 1.

CGH Analysis

APST (n = 9). Only 3 of 9 APSTs showed any chromosomal imbalance. One tumor showed a gain of chromosome 8q and loss of 8p, the second a gain of chromosome 8 and 12, and the third a loss of chromosome 9 (Fig 2A) resulting in an average number of copy alterations (ANCA) of 0.44.

MPSCs (n = 10). The number of chromosomal aberrations in MPSC was higher. Six of 10 showed some chromosomal imbalances. At least 3 sites were affected in 4 of 10 tumors (Table 2). A total of 14 aberrations in 10 tumors were observed, resulting in an ANCA of 1.4. Gains of chromosomes or chromosome arms were observed on chromosome 16p (3 of 10), 1q (2 of 10), 8 (2 of 10), 12 (2 of 10), 2 (1 of 10), 3 (1 of 10), and 5 (1 of 10). Relative losses were observed on chromosome 4 (1 of 10), 9 (1 of 10), and 17 (1 of 10). All changes

TABLE 1. Patient Age and Tumor Stage for 30 Cases of APST, MPSC, and Serous Carcinoma

Case	Age (yr)	Stage	Ovarin Tumor	Implant Type
1	34	IIB	APST	Noninvasive
2	44	IIIB	APST	Noninvasive
3	46	IIIC	APST	Noninvasive
4	15	IIB	APST	Noninvasive
5	37	IIIC	APST	Noninvasive
6	59	IIIB	APST	Noninvasive
7	27	IA	APST	—
8	68	IA	APST	—
9	77	IIA	APST with focal micropapillary architecture*	Noninvasive
10	29	IIIC	MPSC	Invasive
11	48	IIIB	MPSC	Noninvasive
12	35	IB	MPSC	—
13	31	IIA	MPSC	Invasive
14	61	IA/IIB	MPSC with associated APST	Noninvasive
15	31	IA	MPSC	—
16	31	IIIB	MPSC	Invasive
17	51	IIIB	MPSC	Invasive
18	82	IA	MPSC with focal invasion	—
19	47	IIB	MPSC	Noninvasive
20	90	IIB	Serous carcinoma, well differentiated	Invasive
21	80	IIIC	Serous carcinoma, well differentiated	Invasive
22	68	IIIB	Serous carcinoma, moderately differentiated	Invasive
23	35	IIIB	Serous carcinoma, moderately differentiated	Invasive
24	63	IIIB	Serous carcinoma, moderately differentiated	Invasive
25	47	IIB	Serous carcinoma, poorly differentiated	Invasive
26	70	IIIB	Serous carcinoma, poorly differentiated	Invasive
27	51	IIIB	Serous carcinoma, poorly differentiated	Invasive
28	52	IIIB	Serous carcinoma, poorly differentiated	Invasive
29	62	IIIC	Serous carcinoma, poorly differentiated	Invasive
30	81	IIB	Serous carcinoma, poorly differentiated	Invasive

* The micropapillary architecture measured <5 mm; therefore, the tumor did not qualify as MPSC.

affected entire chromosomes or chromosome arms (Fig 2B).

Serous Carcinomas (n = 11). Serous carcinomas showed chromosomal changes in all cases. More than 3 chromosomal imbalances were detected in 9 of 11 cases (Table 2). A total of 113 chromosomal gains or losses were identified in 11 cases, resulting in an ANCA of 10.27, which is more than 7 times as high as in MPSC and more than 23 times as high as in APST. Recurrent gains were found on chromosomes 3q (10 of 11), 8q (7 of 11), 2p (4 of 11), 5p (4 of 11), 12p (3 of 11), and 1q (2 of 11). Frequent losses were seen on chromosomes 16q (7 of 11), 18q (6 of 11), 4q (5 of 11), 9p (5 of 11), 17q (5 of 11), 17p (4 of 11), 11p (4 of 11), and 13 (3 of 11).

Many changes affected parts of chromosome arms or high-level amplifications of small regions (Fig 2C).

Subclassification of Recurrent Chromosomal Aberrations Correlated With Tumor Type

When comparing the chromosomal copy number changes in the 3 categories of tumors, we observed specific patterns of recurrent changes that permitted grouping of the aberrations into 4 classes (Table 3). Changes were considered recurrent if they occurred in at least 3 cases in the study.

Class I aberrations represent the only changes that were detected in APSTs: −8p, +8q, +8, −9, and +12. All changes that were observed in the 3 APSTs with chromosomal imbalances were also seen repeatedly in either MPSCs or frankly invasive serous carcinomas.

Class II aberrations constitute known recurrent aberrations in serous carcinoma that were also observed in MPSCs. These alterations included gains of chromosomes 1q, 2p, 3q, and 5p and losses of chromosomes 4 and 17. An MPSC with focal invasion (the only 1 in the study) showed 2 class II aberrations, and the 1 MPSC with documented lymph node metastasis had 3 class II aberrations.

Class III aberrations constitute recurrent aberrations seen only in frankly invasive serous carcinomas in our study. These include losses of chromosomes 16q, 18, 11p, and 13q.

Class IV aberrations were limited to MPSC and consisted of relative gains of 16p. This was the most frequent change in MPSC, present in 3 of 12 cases. The only detectable aberration in 1 case was a gain in 16p (Fig 3).

DISCUSSION

The cytogenetic findings in this study support the proposal that proliferative serous tumors can be divided into 3 distinct categories: APST, MPSC, and serous carcinoma. In addition, the CGH findings provide clues to possible pathways of serous ovarian carcinogenesis. Substantial differences in the frequency and pattern of chromosomal aberrations were found between APSTs, MPSCs, and serous carcinomas. Three of 9

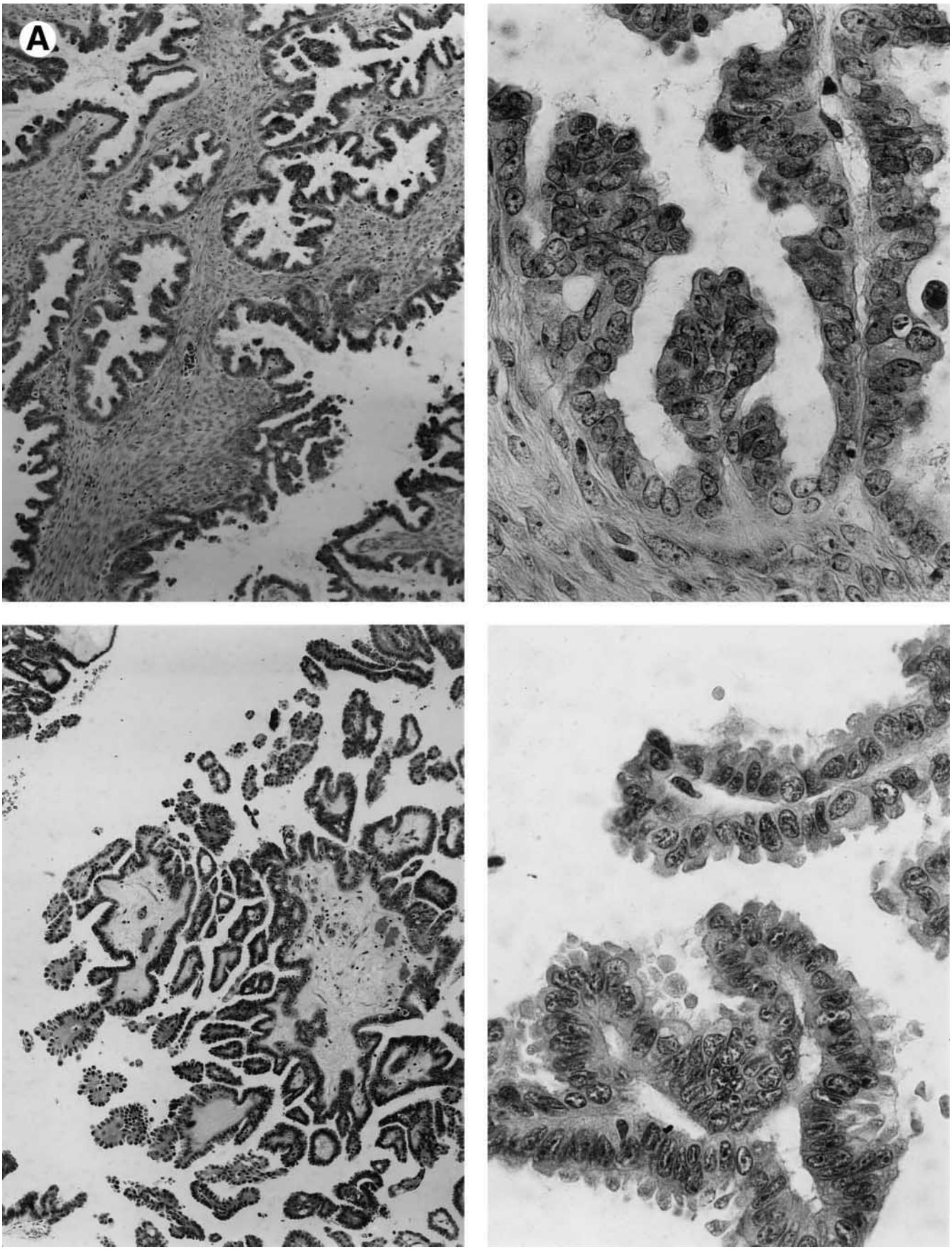

FIGURE 1. Microscopic images. (Original magnifications ×100 and ×630.) (A) Atypical proliferating serous tumors (APST). Hierarchical branching pattern of papillae lined by bland cuboidal to columnar epithelium with focal stratification.

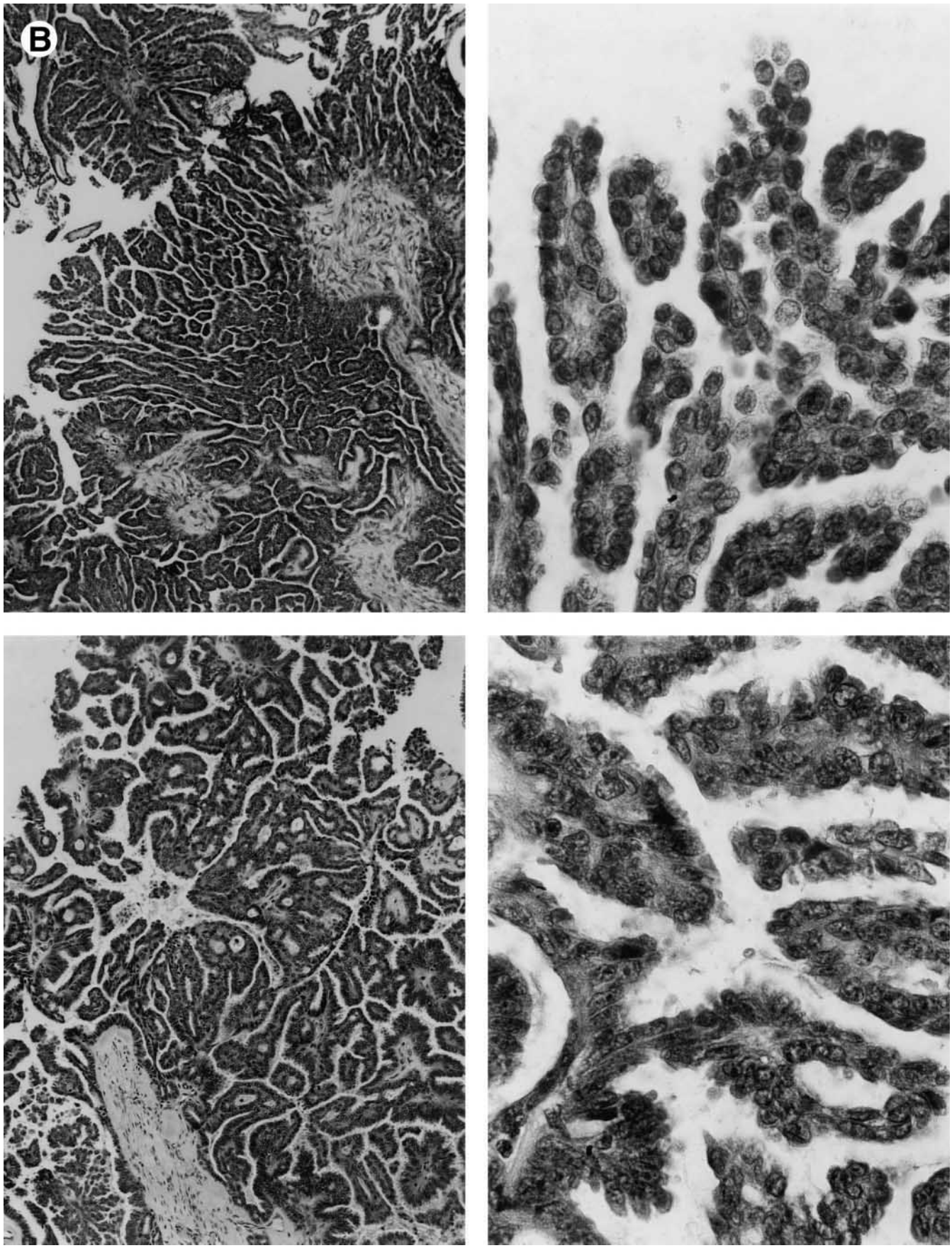

FIGURE 1 (cont'd). (B) Micropapillary serous carcinomas (MPSC). Dense proliferation of micropapillae with minimal stromal support emanating from thick, centrally located papillae.

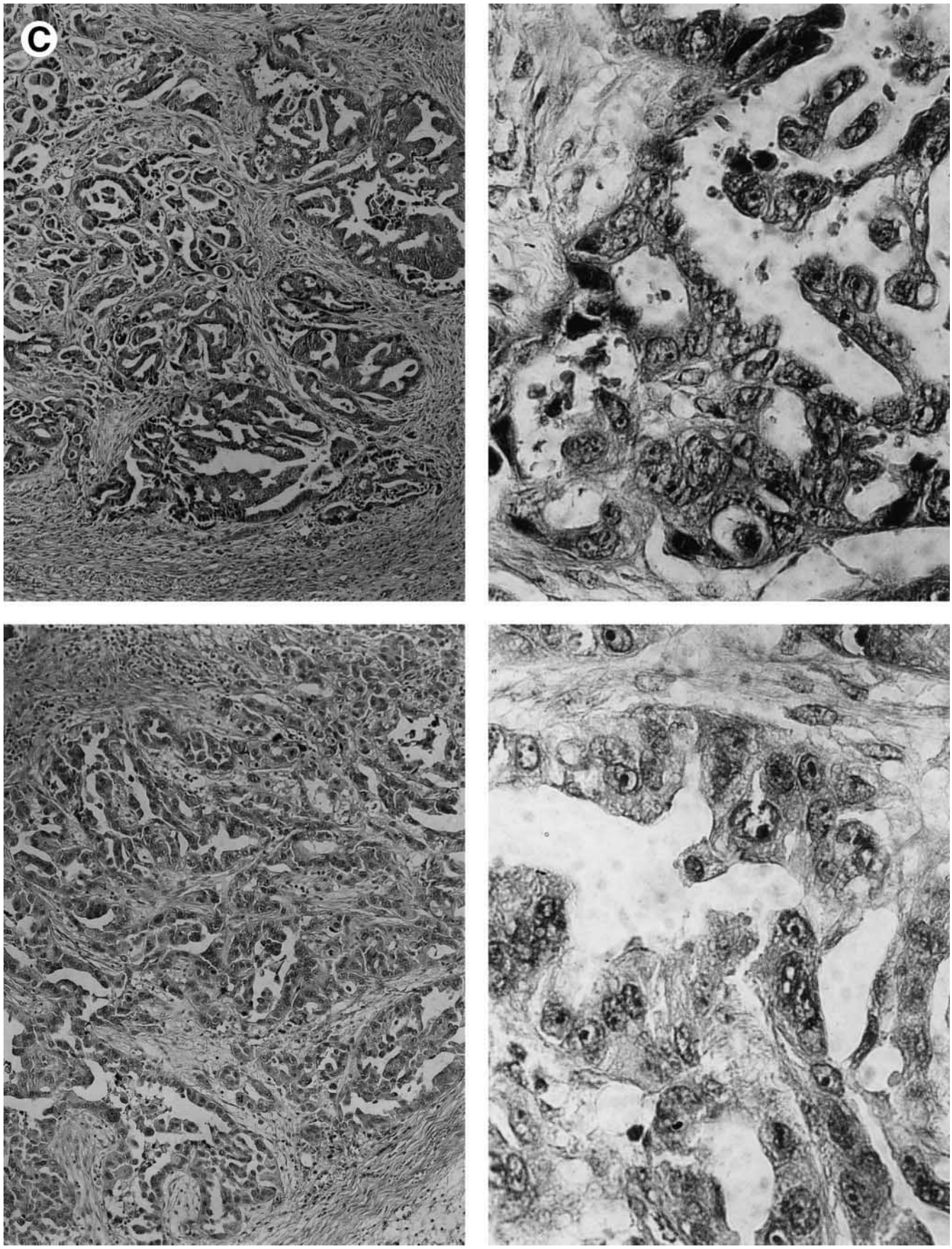

FIGURE 1 (cont'd). (C) Serous carcinomas. Haphazard pattern of invasive nests with slitlike spaces lined by cells with high-grade nuclei.

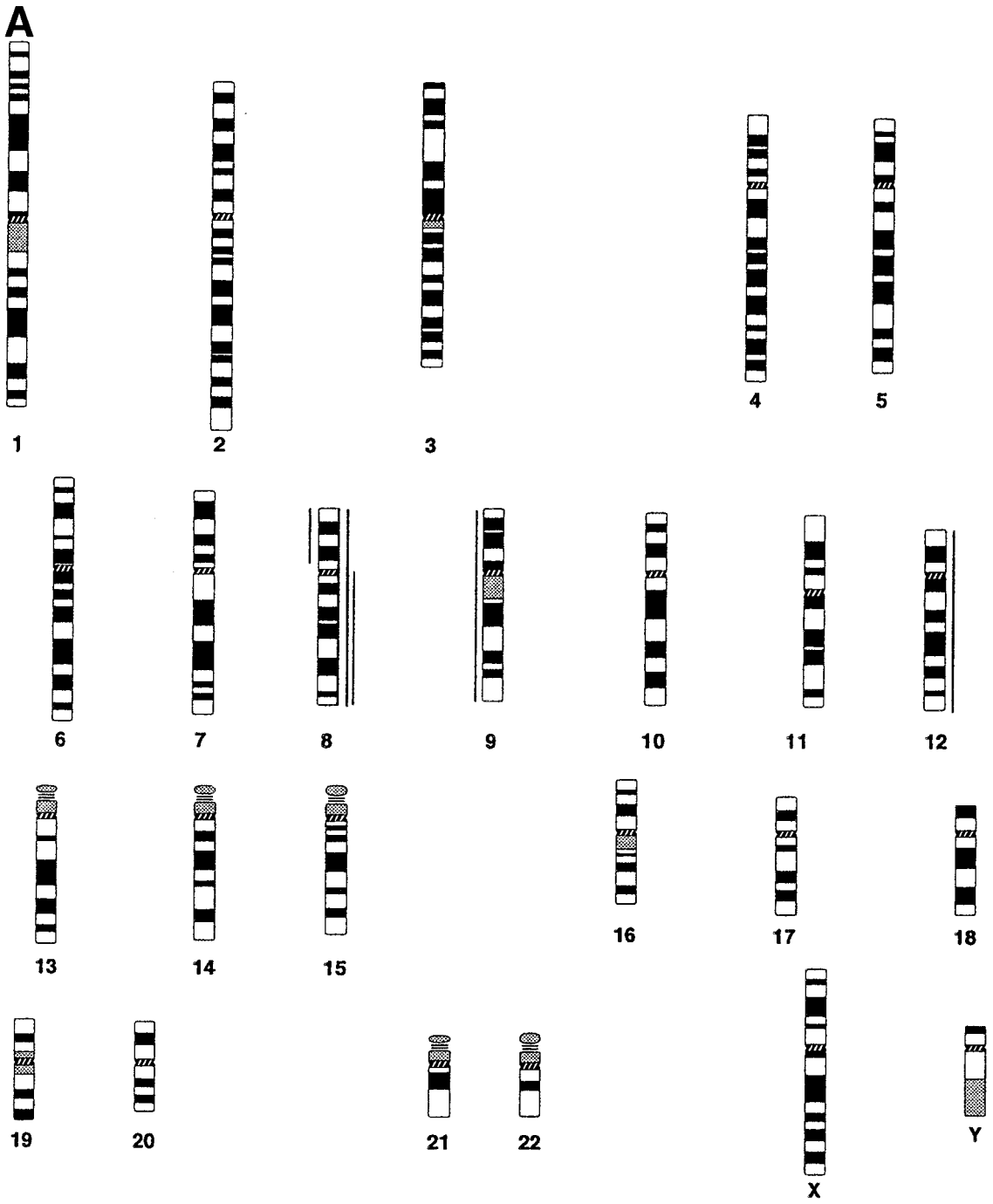

FIGURE 2. Summary ideograms of chromosomal gains (left) and losses (right) seen by CGH in (A) 9 APSTs, (B) 10 MPSCs, and (C) 11 serous carcinomas. Chromosomal regions in which CGH ratios were >1.25 were considered gained, and those in which the ration was <0.75 were considered lost.

APSTs showed some chromosomal imbalances; no tumor had more than 2 changes. In contrast, 6 of 10 MPSCs had imbalances, and 4 of these had at least 3 affected sites. All serous carcinomas showed some chromosomal changes, and 9 of 11 had 3 or more detectable copy number changes.

As expected, the largest number of aberrations was found in serous carcinomas, with an average of 10.3 chromosomal aberrations, compared with 0.44 for APST and 1.44 for MPSC. This difference between serous carcinoma and each of the other 2 groups was statistically significant. Not surprisingly, the difference between APST and MPSC was not statistically significant ($P = .084$) because these 2 entities are indeed closely related. This finding is supported by the fact that mixtures of APST and MPSC frequently occur. Nevertheless, it is striking that MPSCs have a more than 3-fold greater average number of chromosomal aberrations

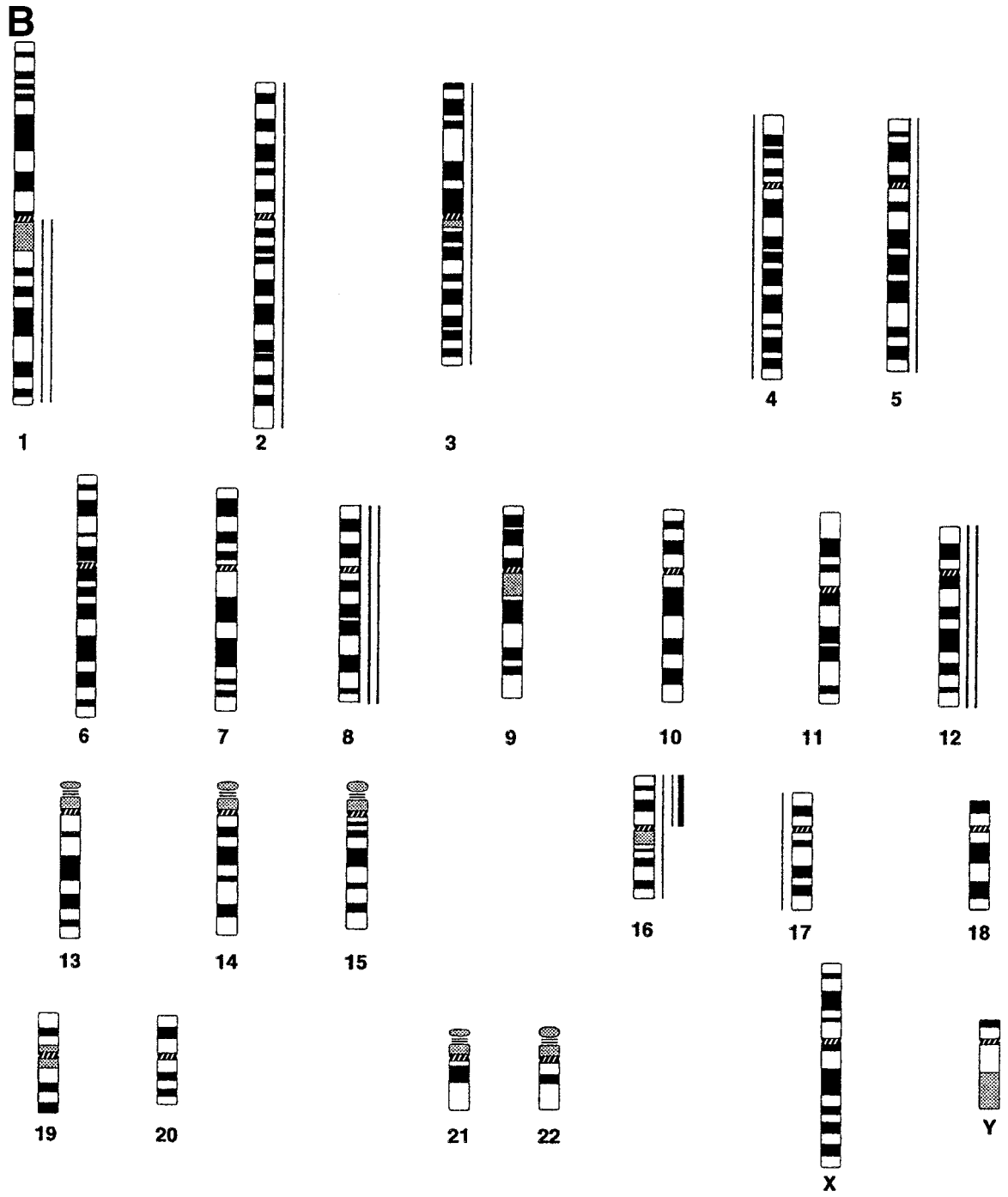

FIGURE 2 (cont'd).

than APSTs. Clearly, more cases need to be analyzed to determine whether this difference is statistically significant.

CGH analysis has been used as a genome-wide screening tool to establish relationships between solid tumors and their precursors on a chromosomal level in a number of sites, including the colon and the uterine cervix.[14-16] Therefore, we attempted to group the aberrations found in our study into classes to facilitate a more comprehensive understanding of tumor progression. Class I changes (copy number changes on chromosome 8, 9, and 12) were present in at least 1 case of all 3 tumor types and therefore most likely represent early changes and suggest that the tumor types are related.

Class II changes were recurrent copy number aberrations seen in serous carcinomas and also in a small number of MPSCs, but not in APSTs. These included

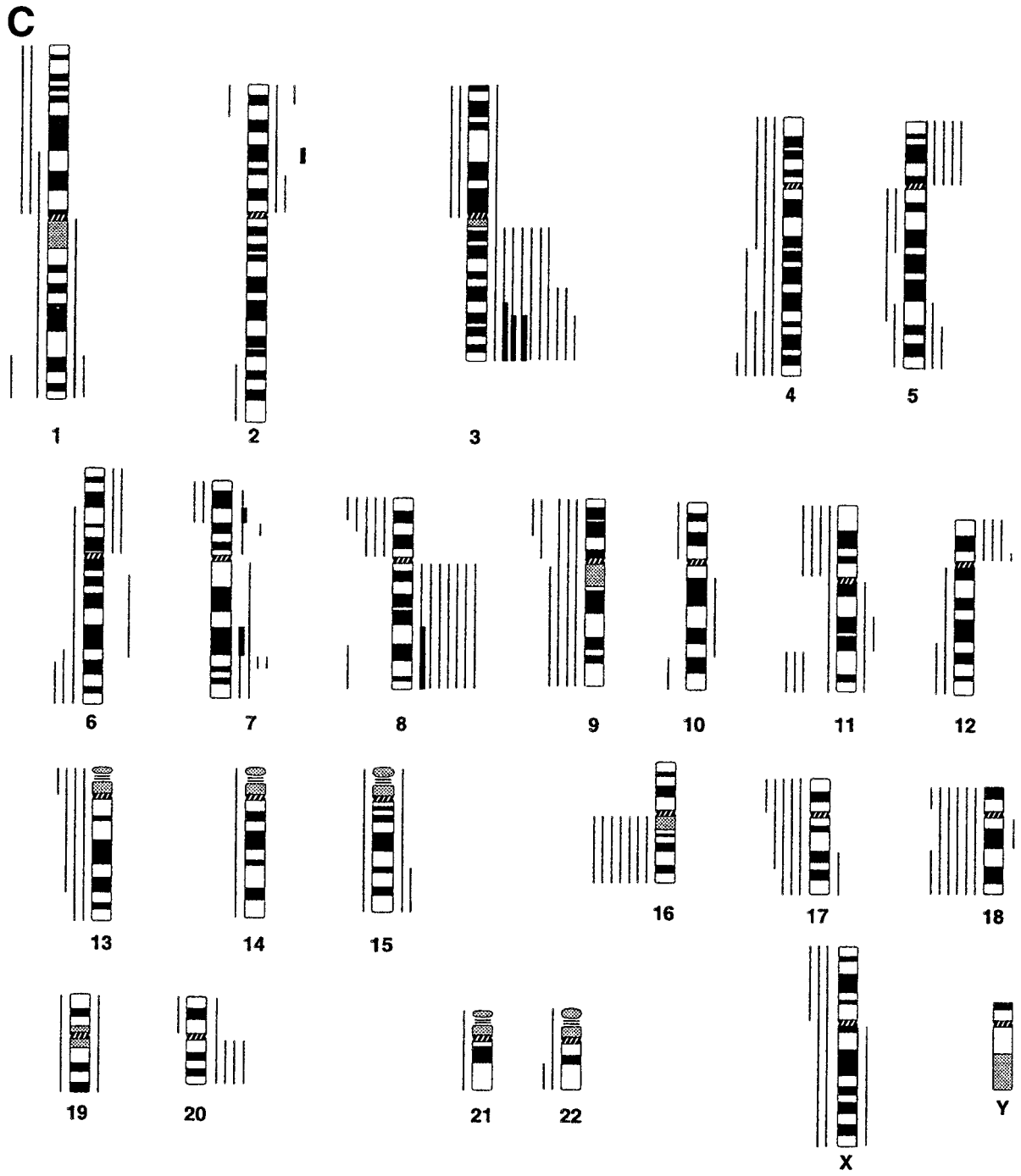

FIGURE 2 (cont'd).

gains of chromosomes 1q, 2p, 3q, and 5p and losses of chromosomes 4 and 17. These changes probably represent the first changes associated with a more aggressive phenotype. All of these changes have been reported previously in other studies of ovarian carcinoma.[6-8] Four of 10 MPSCs had 1 or more aberrations of this type. All 4 MPSCs with class II aberrations had invasive implants, whereas only 1 of 7 MPSCs without class II aberrations had invasive implants, suggesting that these alterations are associated with aggressive behavior.

Class III changes were defined as recurrent alterations present only in invasive serous carcinomas. These included losses of sequences on 18q, 16q, 13q, and 11p. We hypothesize that these changes are associated with an overt malignant phenotype. Under-representation of 11p and 13q was recently reported to be associated with more poorly differentiated ovarian carcinomas.[9]

Class IV changes included gains on chromosome 16p and were found in our series only in MPSCs, suggesting that this alteration represents a distinctive pathway for the development of this tumor. Gain of 16p was

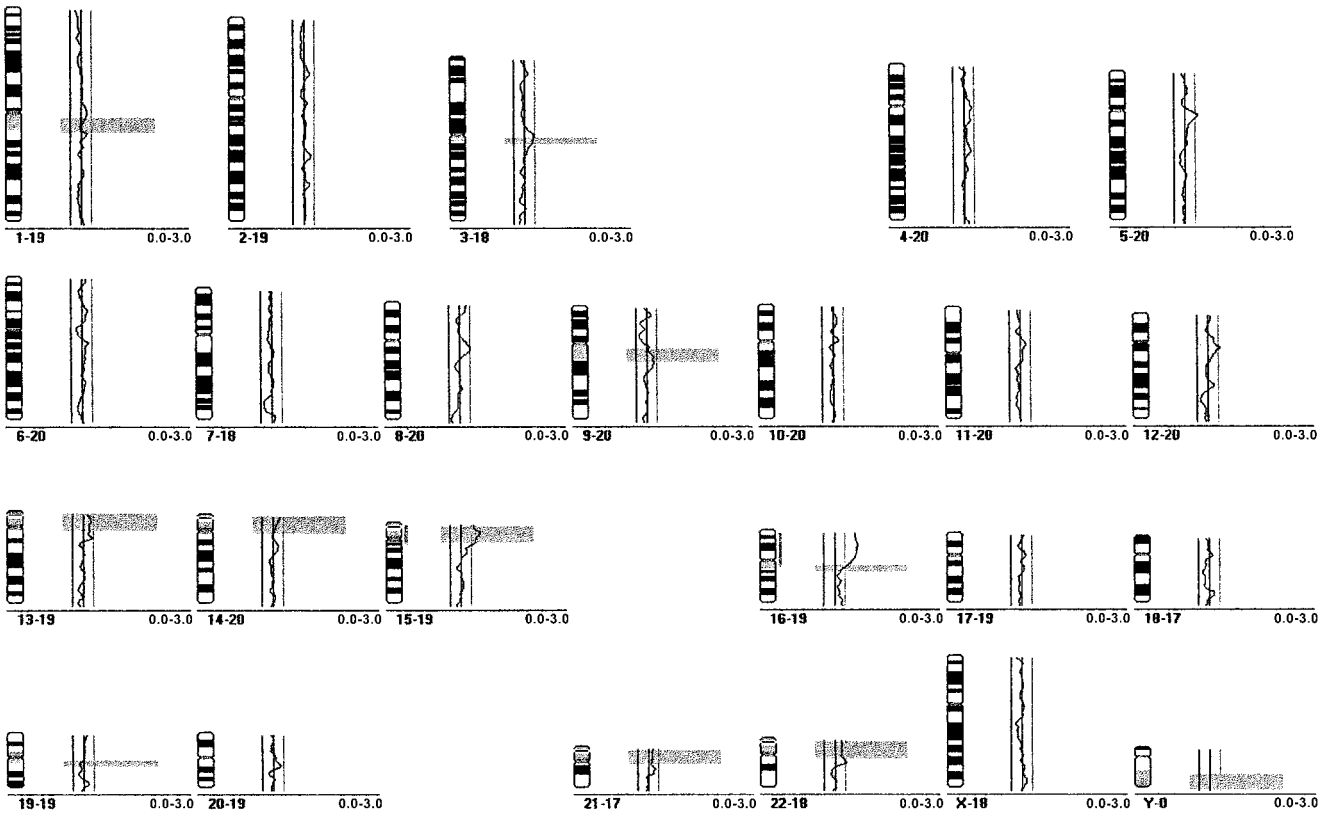

FIGURE 3. CGH profile of MPSC with gain of 16p. Average green-to-red fluorescence ratio profiles calculated from 10 metaphases. For each profile, the black line (middle) indicates a ratio of 1, the green line (right) a ratio of 1.25, and the red line (left) a ratio of 0.75.

the most frequent alteration, seen in 3 of 10 MPSCs. One of these cases appeared to have multiple copies of 16p as the only aberration, further supporting the interpretation that this finding may have an important role in the development of MPSC. Although we did not see this change in any of our cases of invasive serous carcinoma, gains on 16p have been reported in approximately 10% to 33% of ovarian carcinomas in 3 previous studies.[7-9] However, the histologic subtype of the carcinomas was not specified in either of these studies. Therefore, it is possible that they include serous carcinomas with micropapillary features or other types of surface epithelial carcinomas of the ovary, which could explain the difference in our findings. Obviously, further studies are needed to confirm the possible role of amplification of oncogenes on 16p for the development of MPSCs.

The most frequent alteration in all groups was a gain on chromosome 8q. This change is common in many other tumor types in which the smallest region of overlap (SRO) maps to 8q24.1, the site of the *c-myc* gene. The *c-myc* gene has been reported as amplified in 30% of ovarian carcinomas.[17]

Gain of areas on 3q emerged as the most frequent change in serous carcinoma. It was present in 10 of 11 cases of serous carcinoma and 1 of 10 cases of MPSC. This latter case was the only MPSC in the study that presented with lymph node metastasis in addition to widespread intra-abdominal disease with invasive implants. Gain of 3q has been reported in cases of ovarian carcinomas in 3 previous studies, although with much lower frequency.[7-9] However, the histologic subtype of carcinomas was not specified in either of these studies. Gain of 3q has been associated with transition from high-grade cervical dysplasia to invasive squamous carcinoma of the cervix uteri.[14] This aberration has also

TABLE 2. Number of Chromosomal Imbalances in APST, MPSC, and Invasive Serous Carcinomas

	APST	MPSC	Serous Carcinoma
Average number of chromosomal aberrations	0.44	1.4	10.3
Cases with imbalances	3/9	6/10	11/11
Cases with >2 imbalances	0/9	4/10	9/11
Most frequent changes	+8q (2/9)	+16p (3/10)	+3q (10/11)

NOTE. The average number of chromosomal aberrations is statistically different between APST and serous carcinoma ($P < .001$) or MPSC and serous carcinoma ($P < .001$). For the difference between APST and MPSC, the P value is .084.

TABLE 3. Subclassification of Recurrent Chromosomal Aberrations Correlated With Tumor Type

Case	Ovarian Tumor	No. of Aberrations	Class I APST–MPSC–Serous Carcinoma	Class II MPSC–Serous Carcinoma	Class III Serous Carcinoma	Class IV MPSC–Specific	CGH Nonrecurrent
1	APST	1	+8q, −8p				
2	APST	0					
3	APST	2	+8, +12				
4	APST	0					
5	APST	0					
6	APST	0					
7	APST	0					
8	APST	0					
9	APST with local MPSC	1	−9				
10	MPSC	3		+3, +5, −17			
11	MPSC	0					
12	MPSC	0					
13	MPSC	0					
14	MPSC with associated APST	1				++16p	
15	MPSC	3	+8, +12			+16p	
16	MPSC	1		+1q			
17	MPSC	3	+8	+2		+16	
18	MPSC with local invasion	3	+12	+1q, −4			
19	MPSC	0					
20	Serous carcinoma, well differentiated	18	−8p21-pter, −9p21-pter	+1q41-1qter, +2p24-pter, 3q(++3q26), −4q33-ter	−11q23-qter, −13cen-q31, −15q, −18p, −18q21-qter, −20q13-qter		−2q34-qter, +4p, −14, +15q24 - ter, −19, −22q13-qter
21	Serous carcinoma, well differentiated	16	−8p, +8q, −9	+3q26-qter, −p	+3p, −11p, −16p, −18, +20q		+2q31-qter, +6p, +10p, −12q22 - qter, +17q22 - qter, −20p
22	Serous carcinoma, moderately differentiated	8	+8q, −9	+1q, +3q	−18, +20q, −X		+6p
23	Serous carcinoma, moderately differentiated	13	+12p, −9q	+3q24-qter, −4, −17	−6p212-qter, −11, −16q, −18, −X		−1p, −7p15-pter, −15, −22
24	Serous carcinoma, moderately differentiated	9	+8q, −8p	+3q36-qter, −4q-qter, +5p, −17	++8q23-qter, −16q, −17, −18		+5q32-qter, +7p14, −21
25	Serous carcinoma, poorly differentiated	15	−9p	++2p16, +3q(++3q25-qter), −4pter-q22, −4q31-qter, +5p, −17p-q23	−6q24-qter, −11p, −11q23-qter, −13, −16q, −18		−1p22-qter, −3p, −5qcen-5q15, −5q31-5qter, −7p15-pter, −8pter-p22, −8q23-qter, +10q25-qter, −10q21-10q24, +Xq
26	Serous carcinoma, poorly differentiated	12	+ 8q, +12p, −9	+3q24-qter(++3q26), −17	−6q23-qter, +7p(++7p15), −11q23-qter, −13, −16q		+11q13-11q22
27	Serous carcinoma, poorly differentiated	1	+8q				
28	Serous carcinoma, poorly differentiated	2		+3q	+7q		
29	Serous carcinoma, poorly differentiated	4	+8q	+2p, +3q, +5p			
30	Serous carcinoma, poorly differentiated	15	++8q, +12p	+2p, +3, −4, +5p	+7q32, +11q, −11p, −16q, +20		−1p, +5q31-qter, +15, +19

NOTE. Subclasses of recurrent chromosomal aberrations: class I, recurrent aberrations present in APST and MPSC and/or serous carcinoma; class II, recurrent aberrations present in both serous carcinoma and MPSC; class III, recurrent aberrations present only in serous carcinoma; class IV, recurrent aberrations present only in MPSC.

been found in small-cell carcinomas of the lung[18] and in squamous cell carcinoma of the head and neck,[19] where it has been documented as an independent prognostic feature.[20] Three of our cases showed even high-level amplification within the shortest region of overlap encompassing the band of 3q26, providing further support for the hypothesis that important target genes are located in this region. PIK3CA, which encodes the p110α-catalytic subunit of phosphatidylinositol 3-kinase, and eIF-5A2, a eukaryotic initiation factor, are candidate oncogenes from this region that have been previously implicated in ovarian cancer.[21,22]

The low frequency of aberrations in atypical proliferative tumors is in keeping with the results of previous cytogenetic studies on borderline tumors. One study using traditional cytogenetics found aberrations

in 5 of 14 borderline tumors. Two of 3 serous tumors in this study showed trisomy 12, and 1 showed trisomy 8.[10] Relative gains in chromosomes 8 and 12 were also observed in our study and were present in all 3 tumor groups. Wolf et al[12] used CGH to analyze a group of serous borderline tumors. Ten tumors in 9 patients (1 recurrence) were analyzed, and aberrations were found in only 3 of the 10 cases. In concordance with our findings, 2 of the tumors showed a relative gain in chromosome 8q, among other changes that had previously been reported for ovarian carcinoma.[12] A more recent study also reported a gain of 8q and chromosome 12 among the most frequent changes in borderline tumors.[13]

This study has a number of limitations that have bearing on the current analysis and on further studies. It is obvious that a larger number of cases needs to be analyzed for each of the tumor types. Furthermore, because this study was retrospective and involved genetic analysis, preservation of patient anonymity was required. Although we were able to obtain staging information for all patients, we could not obtain clinical follow-up data. Accordingly, we view this as a preliminary study comparing the different subcategories of serous tumors of the ovary. In the future, we plan to analyze invasive and noninvasive implants of both APSTs and MPSCs and correlate the cytogenetic changes with the primary tumor and the clinical outcome. In addition, it is important to emphasize that CGH is a screening test and that certain genetic changes such as small deletions are below the level of resolution of CGH.[23] One study correlating LOH and CGH data in a group of ovarian carcinomas found that only 31% of cases showing LOH were attributable to deletions detected by CGH.[6] Furthermore, microsatellite instability, which would not change the CGH profile, has recently been reported in a number of borderline tumors. In fact, studies analyzing tumors of the colon and endometrium show that microsatellite instability is found more frequently in tumors that have normal CGH profiles.[8,24]

In summary, this study identified chromosomal aberrations in MPSCs of the ovary that are shared with both APSTs and invasive serous carcinomas; others were found only in serous carcinomas. These findings suggest a progression from APST via MPSC to serous carcinoma for a subgroup of tumors. Other changes were observed only in MPSCs, suggesting that some MPSCs develop independently, apart from APSTs and serous carcinoma. Finally, we identified several cytogenetic changes that could represent prognostic risk factors, such as increasing numbers of chromosomal changes (>2) and the presence of individual changes that are recurrent and characteristic of serous carcinoma. Further studies with larger numbers of cases and long-term clinical follow-up are needed to determine whether these findings have potential applications in the diagnosis and treatment of serous tumors of the ovary.

REFERENCES

1. Burks RT, Sherman ME, Kurman RJ: Micropapillary serous carcinoma of the ovary. Am J Surg Pathol 20:1319-1330, 1996

2. Seidman JD, Kurman RJ: Subclassification of serous borderline tumors of the ovary into benign and malignant types. A clinicopathologic study of 65 advanced stage cases. Am J Surg Pathol 20: 1331-1345, 1996

3. Eichhorn JH, Bell DA, Young RH, et al: Ovarian serous borderline tumors with micropapillary and cribriform patterns. A study of 40 cases and comparison with 44 cases without these patterns. Am J Surg Pathol 23:397-409, 1999

4. Kallioniemi A, Kallioniemi O-P, Sudar D, et al: Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 258:818-821, 1992

5. Mitelman F: Catalog of Chromosome Aberrations in Cancer. New York, NY, Wiley-Liss, 1994

6. Iwabuchi H, Sakamoto M, Sakunaga H, et al: Genetic analysis of benign, low-grade, and high-grade ovarian tumors. Cancer Res 55:6172-6180, 1995

7. Arnold N, Hägele L, Walz L, et al: Overrepresentation of 3q and 8q material and loss of 18q material are recurrent findings in advanced human ovarian cancer. Genes Chromosomes Cancer 16:46-54, 1996

8. Sonoda G, Palazzo J, du Manoir S, et al: Comparative genomic hybridization detects frequent overrepresentation of chromosomal material from 3q36, 8q24, and 20q13 in human ovarian carcinomas. Genes Chromosomes Cancer 20:320-328, 1997

9. Kiechle M, Jacobsen A, Schwarz-Böger U, et al: Comparative genomic hybridization detects genetic imbalances in primary ovarian carcinoma as correlated with grade of differentiation. Cancer 91:534-540, 2001

10. Pejovic T, Iosif CS, Mitelman F, et al: Karyotypic characteristics of borderline malignant tumors of the ovary: trisomy 12, triosomy 7 and r(1) as nonrandom features. Cancer Genet Cytogenet 92:95-98, 1996

11. Diebold J, Deisenhofer I, Baretton GB, et al: Interphase cytogenetic analysis of serous ovarian tumors of low malignant potential: Comparison with serous cystadenomas and invasive serous carcinomas. Lab Invest 75:473-485, 1996

12. Wolf NG, Abdul-Karim FW, Farver C, et al: Analysis of ovarian borderline tumors using comparative genomic hybridization and fluorescence in situ hybridization. Genes Chromosomes Cancer. 25: 307-315, 1999

13. Blegen H, Einhorn N, Sjövall K, et al: Prognostic significance of cell cycle proteins and genomic instability in borderline, early and advanced stage ovarian carcinomas. Int J Gynecol Cancer 10:477-487, 2000

14. Heselmeyer K, Schröck E, du Manoir S, et al: Gain of chromosome 3q defines the transition from severe dysplasia to invasive carcinoma of the uterine cervix. Proc Natl Acad Sci USA 93:479-484, 1996

15. Ried T, Knutzen R, Steinbeck R, et al: Comparative genomic hybridization reveals a specific pattern of chromosomal gains and losses during the genesis of colorectal tumors. Genes Chromosomes Cancer 15:234-245, 1996

16. Ried T, Heselmeyer-Haddad K, Blegen H, et al: Genomic changes defining the genesis, progression and malignancy potential in solid human tumors: A phenotype/genotype correlation. Genes Chromosomes Cancer 25:195-204, 1999

17. Berchuck A: Biomarkers in the ovary. J Cell Biol 23:223-226, 1995

18. Ried T, Petersen I, Holtgreve-Grez H, et al: Mapping of multiple DNA gains and losses in primary small cell lung carcinomas by comparative genomic hybridization. Cancer Res 54:1801-1806, 1994

19. Brzoska PM, Levin NA, Fu KK, et al: Frequent novel DNA copy number increase in squamous cell head and neck tumors. Cancer Res 55:3055-3059, 1995

20. Bockmühl U, Schlüns K, Küchler I, et al: Genetic imbalances with impact on survival in head and neck cancer patients. Am J Pathol 157:369-375, 2000

21. Shayesteh L, Lu Y, Kuo W, et al: PIK3CA is implicated as an oncogene in ovarian cancer. Nat Genet 21:99-102, 1999

22. Guan X-Y, Sham J, Tang T-M, et al: Isolation of a novel candidate oncogene within a frequently amplified region at 3q26 in ovarian cancer. Cancer Res 61:3806-3809, 2001

23. du Manoir S, Schröck E, Bentz M, et al: Quantitative analysis of comparative genomic hybridization. Cytometry 19:27-41, 1995

24. Schlegel J, Stumm G, Scherthan H, et al: Comparative genomic in situ hybridization of colon carcinomas with replication error. Cancer Res 55:6002-6005, 1995